STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG
Jack, Lulu and Sam Willson
Professor of Biochemistry

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Dr. Michael Stoker
Imperial Cancer Research Fund Laboratories
P.O. Box 123
Lincoln's Inn Fields
London, England

Dear Michael:

Your secretary wrote me of your visit to the States; I hope that you feel welcome enough at Stanford to let me know when you're within striking distance of Palo Alto so that we can arrange for a visit. We've come a long way since your last visit but more importantly we'd love to see you again.

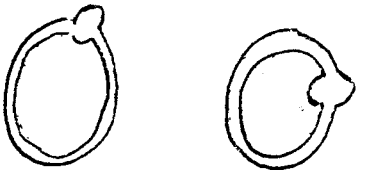
It's really marvelous that you're able to resurrect more of the abortives and stables and to send us cells for the DNA preps. We have trypsinized the cells, quenched with serum and washed 1 time by centrifugation with Tris-saline and then frozen the cell pellets. If we got the cells you indicated that way it would be great. With respect to ST-1, we are repeating the determination with another batch of DNA and we'll do it with your cells as well; that should give a pretty definitive answer. As you say, if they remain transformed without having the viral DNA that would be interesting. Do you have anti PY-T serum? It could be important to know if ST-1 is T-ag$^+$ or T-ag$^-$.

I spoke to Helene Smith last week and she passed on some additional information. The hybridizable SV40 DNA in one of their isolates sediments with the cell DNA in an alkaline sucrose gradient done according to Sambrook et al. She can detect "no" infectious SV40 DNA in such cells. Presumably then it is "integrated". But in one of their clones, the one which gave variable amounts of SV40 sequences in different clonal isolates of the original abortive, and on different trials, contained no SV40 DNA after growing up a large batch of cells. Very strange indeed. The abortives that contain DNA show about 1% of the transforming efficiency of normal 3T3 with wild-type SV40 DNA virus. Didn't you find that BHK abortives could be transformed at normal efficiencies when challenged with PY?

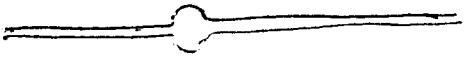
We plan to make highly labeled P^{32}-SV40 DNA for use in detecting SV40 sequences. The procedure would be the same as for PY; purified SV40 Form I is used as template instead of PY I in the DNA polymerase reaction and that generates that reagent for annealing. We could test Warner's clones when they're ready.

We've just had another set of interesting results you might like to

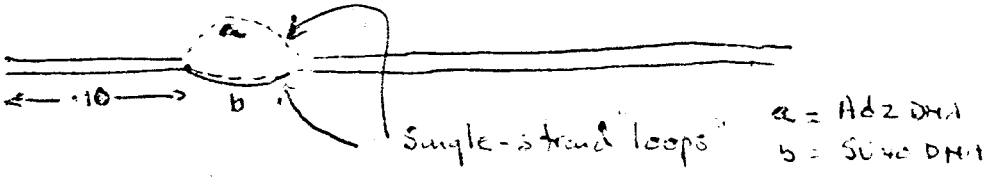
hear. One of my students, John Morrow , has been studying the action of the restriction enzyme, R_I (coded for by the Resistance Transfer Factor I, RTF_I, carried by E. coli) on SV40 DNA. This enzyme has been purified by Herb Boyer in SF who gave us some for these experiments. R_I cleaves SV40 Form I DNA (prepared from plaque purified virus) quantitatively to unit length linear molecules; the distribution of lengths (as seen in EM) is within 2% that of the circles. Each single strand is unit length in alkali so quite clearly the enzyme makes only a double-strand cleavage and no more. The molecules are unique; when denatured and then renatured they produce only linear molecules with the same length distribution as the starting molecules. Had they been circularly permuted linears or even two or several types such denaturation-renaturation produces circular molecules (the result found with linears produced by P_I or B-restriction enzymes or with DNA'ase). Further proof of the uniqueness of the break is the following: Delius, at CSH, has found that T_4 phage gene 32 protein binds to SV40 DNA Form I and if fixed to the DNA with glutaraldehyde and then spread on grids for EM inspection one obtains molecules with a single " bubble."



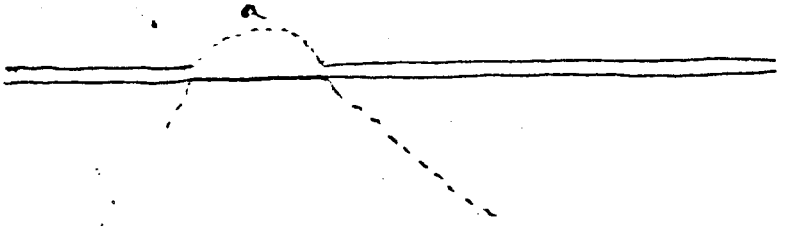
If such molecules are treated with R_I one obtains linear molecules with a bubble at only one location;



about 40% in from one end. Thus we can differentiate one end from the other. Most recently, John has done the following; if Ad_2-SV40 ND_1, the non-defective hybrid of Ad 2 and SV40 DNA (which, according to Lewis contains the "U-ag gene" of SV40 covalently integrated into the Ad 2 chromosome) is denatured and renatured with wild-type Ad 2 DNA one can observe the following heteroduplex in the EM.



There are two single-stranded (indicated as dashed lines) loops about 16% in from one end; one segment (a) is longer than the other. If SV40 R_I produced linears are included in the denaturation-renaturation mixture and the product is spread on grids one sees another picture:



The shorter segment of the loop now appears double stranded and from it comes two single-stranded tails. The length of the tails and the paired loop is about equal to an SV40 length. Thus the R_I restriction enzyme makes a break near the U-ag gene. There is some reason to believe from other Ad 2-ND viral DNA's that the TSTA and T-ag genes are on the longer arm of the SV40 segment.

I'd like very much to come to London for a bit in September . If you're going to be there during the first three weeks or so it would be fun to come over and try to do some experiments. Will you be moved into the new labs then or is that just about the time you'll be moving in? I'll keep in touch with you about how things are going on this and other projects. If you know of any appropriate conferences or meetings in England or on the continent in the Fall, please let me know.

So much for now. My best to all at ICRF and to Veronica. Peter Beard is doing very well and I'm delighted at his being here. He's made several very interesting findings that are proving very useful; he sends his regards to all.

Sincerely,

Paul

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